

REMARKS

In this Amendment, Applicant has cancelled Claim 25, without prejudice or disclaimer, amended Claims 10 and 14. Claims 10 and 14 have been amended to further specify the invention. It is respectfully submitted that no new matter has been introduced by the amended claims. All claims are now present for examination and favorable reconsideration is respectfully requested in view of the preceding amendments and the following comments.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH:

Claims 10 and 12 – 13 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the invention was filed, had possession of the claimed invention.

It is respectfully submitted that in view of presently claimed invention, the rejection has been overcome. In particular, Claim 10 has been amended to limit to “reducing” the inhibition of endogenous 13-HODE synthesis, which has sufficient support in the specification. In addition, rejections to Claims 12 and 13 have been overcome due to their dependency on Claim 10.

Accordingly, withdrawal of the rejection under 35 U.S.C. § 112 first paragraph is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH:

Claims 10 and 12 – 13 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

It is respectfully submitted that in view of presently claimed invention, the rejection has been overcome. In particular, Claim 10 has been amended to specify that “an effective amount of” an omega-3 fatty acids formulation comprising 13-HODE is administered to the subject. In addition, rejections to Claims 12 and 13 have been overcome due to their dependency on Claim 10.

Accordingly, withdrawal of the rejection under 35 U.S.C. § 112 second paragraph is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 102:

Claims 14 – 15, 18 – 20 and 23 have been rejected under 35 U.S.C. § 102 (a) as allegedly being anticipated by Carlsson et al. (WO 99/44585), hereinafter Carlsson. Examiner states that since Claims 19 and 20 are not limited to isolated or purified forms of fatty acids, the composition in Carlsson contains Evening Primrose oil (gamma linolenic acid and linolenic acid) and therefore anticipates the claimed composition. Applicant respectfully submits that the present-claimed invention is not anticipated by the cited reference.

It is respectfully submitted that the newly amended Claim 14 has included the limitation that “at least one omega-3 fatty acid” is “selected from the group consisting of EPA, DHA, a derivative of EPA and a derivative of DHA.” Carlsson does not teach or suggest that the omega-3 fatty acid is selected from the group consisting of EPA, DHA, a derivative of EPA and a derivative of DHA, which is required in the present invention. In addition, rejections to Claims 15, 18 – 20 and 23 have been overcome due to their dependency on Claim 14.

Accordingly, withdrawal of the rejection under 35 U.S.C. § 102 is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 103:

Claims 16 – 17 and 21 – 22 have been rejected under 35 U.S.C. § 103, as allegedly being obvious and unpatentable over Streber (US 5,102,912), hereinafter

Streber, in view of Carlsson. Claims 10, 12 – 13 and 25 – 26 have been rejected under 35 U.S.C. § 103, as allegedly being obvious and unpatentable over Miller et al. (J. Invest Dermatol, 1990, 94:353-358), hereinafter Miller.

It is respectfully submitted that in view of presently claimed invention, the rejection has been overcome. The newly amended Claim 14 has included the limitation that “at least one omega-3 fatty acid” is “selected from the group consisting of EPA, DHA, a derivative of EPA and a derivative of DHA.” Neither Carlsson nor Streber teaches or suggests that co-administration of 13-HODE with EPA, DHA, a derivative of EPA or a derivative of DHA, which is required in the present invention. There is no motivation to combine these two references. Even if they are combined, they do not disclose the invention as presently claimed in Claims 16 – 17 and 21 – 22.

Applicant respectfully disagrees with Examiner on the teaching of Miller reference that “one having ordinary skill in the art would have expected that the co-administration of 13-HODE with omega-3 fatty acids (e.g. EPA and DHA) would be advantageous over the administration of the omega-3 fatty acid alone.” To the contrary, it is respectfully submitted that Miller teaches away from the combination of 13-HODE with EPA or DHA. Miller discloses that the treatment with EPA or DHA exacerbates epidermal hyperproliferation, hypergranulosis and hyperkeratosis. This is the Miller model of essential fatty acid deficiency-induced hyperproliferation dermatosis. Miller teaches hyperproliferation, hypergranulosis and hyperkeratosis. However, Miller does not teach the combination of 13-HODE and EPA or DHA, nor suggest that it is desirable to prepare such combination. One of ordinary skill in the art would not be motivated by Miller to prepare a combination of 13-HODE and EPA or DHA for the treatment of clinical dermatitis. It is beyond the expectation of one of ordinary skill in the art that a topical application of EPA or DHA in combination with 13-HODE would be beneficial for treatment of clinical dermatitis. To the contrary, one skilled in the art would instead expect that a topical application of EPA or DHA in combination with 13-HODE would worsen any clinical dermatitis because any beneficial effects of 13-HODE would be masked by the presence of EPA and DHA in the combination.

Applicant respectfully submits for Examiner’s reference an article entitled “Effects of Linoleic Acid Supplements on Atopic Dermatitis” by Gimenez-Amau et al

(1997), hereinafter Gimenez-Amau, which indicates that it would not have been obvious at the date of the present invention to prepare a combination of 13-HODE and EPA or DHA.

Gimenez-Amau discloses that topical treatment of 13-HODE ameliorates the extent of spread of symptomatic atopic eczema, whereas topical treatment with EPA or DHA has no beneficial effect (see Table 1). In addition, all patients treated with EPA and DHA withdrew from the study because of significant adverse effects (See "Clinic results" on pages 5 – 6). Therefore, one having ordinary skill in the art would not expect 13-HODE in combination with EPA or DHA would perform better than use of 13-HODE alone.

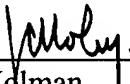
Accordingly, Applicant respectfully requests that the rejection under 35 U.S.C. §103 be withdrawn.

Having overcome all outstanding grounds of rejection, the application is now in condition for allowance, and prompt action toward that end is respectfully solicited.

Respectfully submitted,

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EFFECTS OF LINOLEIC ACID SUPPLEMENTS ON ATOPIC DERMATITIS

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SUMMARY

Fish oil [eicosapentaenoic (EPA) + docosahexaenoic (DHA) acids] and vegetable oil [linoleic acid (LA)] supplements are thought to be beneficial in the treatment of severe atopic dermatitis (AD). [EPA+DHA] would compete with arachidonic acid for the cyclo- and lypoxigenase enzymes decreasing synthesis of pro-inflammatory prostanooids and leukotrienes. If AD is a fatty acid deficiency related disease, LA should be more beneficial than [EPA+DHA]. This study is a pilot, placebo-controlled, randomized, double-blind clinical trial: 48 patients with severe AD were included. Patients ingested either LA (3 gr/day), [EPA+DHA] (2.0+1.3 gr/day) or placebo (3.0 gm/day, oleic acid) for 12 weeks. AD severity was measured by Rajka score (≥ 7) and the Rule of 9. An histomorphometric assessment and the study of mRNA ICAM-1 / IL-10 expression by *in situ* hybridization pre and post treatment was performed. Epidermis/dermis 5- 12- and 15- hydroxyeicosatetraenoic acid (HETEs) and 13-hydroxy octadecadienoic acid (13-HODE) levels in skin biopsies were measured as indices of [EPA+DHA] and LA skin uptake and metabolism, using reverse phase HPLC. There was no differences in AD severity among the three groups before treatment. Rajka and Rule of 9 correlation was $r = 0.9659$. Significant differences in the % of Rajka score and Rule of 9 reduction between patients treated with LA and [EPA+DHA] ($p < 0.0001$) and also in patients treated with LA and placebo ($p < 0.0001$) were obtained. Histomorphometry showed significant differences in the % epidermal area reduction, between LA and EPA+DHA treated patients ($p < 0.005$) and between LA and placebo ($p < 0.011$). *In situ* hybridization showed a marked decreased of ICAM-1 and IL-10 expression after treatment with LA. We observe higher levels of 13-HODE than 15-HETE with significant differences in healthy skin ($p < 0.016$) and sick skin ($p < 0.015$). Clinical and pathological improvment could not be statistically correlated with the increase levels of 13-HODE observed in healthy skin in AD patients. AD is an essential fatty acid related disease and it can be ameliorated with LA supplementation.

INTRODUCTION

Atopy is primarily a condition of hypersensitivity which can lead to a disease. Atopic disease include atopic eczema, allergic bronchial asthma or hay fever. Atopy lead in many cases to a syndrome of different diseases including respiratory, gastrointestinal and skin symptoms.⁽¹⁾

Atopic dermatitis or atopic eczema is a widely prevalent disease, specially in childrens. The main signs and symptoms are dry itching skin, and eczematous inflammation. Its etiopathophysiology is complex and let us to consider the most frequently concepts discussed in atopic eczema. These are: genetics, trigger factors as aeroallergens, superantigens or stress, an immunological dysfunction characterized by Th2 lymphocytes cytokine profile (IL-4, IL-5, IL-10), hyper IgE, the increase activity of monocitary phosphodiesterase, an impaired epidermal barrier function and finally, an abnormal linoleic acid metabolism characterized by the defective activity of Δ6 desaturase.

Fish oil [eicosapentaenoic (EPA) + docosahexaenoic (DHA) acids] and vegetable oil [linoleic acid (LA)] supplements are thought to be beneficial in the treatment of severe atopic dermatitis (AD).

EPA an n-3 unsaturated fatty acid (20:5) is transformed by lipoxygenase to leukotriene (LT) A5 and LTB5 or by cyclooxygenase to prostaglandin (PG) PGE5, PGI3 and thromboxane (TX) TXA3. These compounds have been shown to have lower inflammatory activity than the PGs of the 2 or the LTs of the 4 series, respectively.^(2,3)

The n-6 fatty acids, specially the combination of linoleic acid (LA) plus gammalinolenic acid (GLA) have been implicated in multiple biological functions as immunomodulation, epidermal proliferation, epidermal barrier function, carcinogenesis or atherosclerosis. The exact mechanism of action is unknown. Multiples clinical trials support the efficacy of LA+GLA in the treatment of atopic dermatitis.⁽⁴⁻⁹⁾ However, the efficacy of this treatments is still doubtful for some people.⁽¹⁰⁾ The biological relevance of essential fatty acids is demonstrate by the existence of the "Essential Fatty Acid deficiency syndrome", characterized in mice by dry skin and dermatitis, weight loss, cardiomegalias, loss of fertility or cholesterol deposit in lungs.⁽¹¹⁾

The aim of this study was to demonstrate that atopic dermatitis is initially, also an essential fatty acid deficiency related disease. The n-6 fatty acids (LA) should be more effective than n-3 (EPA+DHA) fatty acids to treat severe atopic dermatitis. Clinical benefit should correlate well with pathological, immunological and biochemistry findings.

STUDY DESIGN & METHODS

Study design

Forty eight adult patients with chronic and severe atopic dermatitis (Rajka score ≥ 7) were randomly included in a pilot, parallel group, double blind, placebo controlled clinical trial. The clinical trial was previously approved by the ethical committee in our hospital and by the Health Ministry in Spain (nº 91/115). All patients included signed the informed consent. The study was performed during 1992-1993. A one month wash out period preceded the patient

inclusion. Any other treatment was permitted during the clinical trial. Patients ingested either LA (3 gr/day), EPA +DHA (2.0+1.3 gr/day) or placebo (3.0 gr/day, oleic acid). Hard gelatine capsules, size 0 were employed. The semisolid oil content had a fusion point at 35°C -40°C.

Evening-primrose oil or borage oil, both vegetable oils, rich in linoleic acid with different percentage of GLA (8-11% for evening-primrose and 18-25% for borage oil) are very well known and commonly used as dietary supply or in topical creams in atopic dermatitis. In this study we used as source of linoleic acid sunflower oil. It was provided by Serra Pamies S.A (Reus, Spain). The porcentual composition of this sunflower oil was: linoleic acid 62.60, oleic acid 24.51, palmitic acid 6.42, estearic acid 4.45, behenic acid 0.62, lignoceric acid 0.27, arachidic acid 0.26, gadoleic acid 0.15, palmitoleic acid 0.09, miristic acid 0.06, margaric acid 0.05, linolenic acid 0.05, margaroleic acid 0.05, others 0.47. Each capsule of sunflower oil contained 250 mg of LA in triglyceride form. Twelve capsules per day were ingested during three months.

We chose sunflower oil rich in LA and poor in GLA because we measured a direct metabolite from LA by the 15-lipoxygenase pathway, 13-HODE. This LA metabolite has a proved antiproliferative capacity.⁽¹²⁾

Clinic evaluation

The clinic evaluation was done, in forty eight patients, before to initiate the treatment, at 6 weeks and at 12 weeks with treatment. The evaluation was performed always by two medical doctor and was blind. We use as score of severity the "Rajka score"⁽¹³⁾ that consider the parameters: extension, course of eczema and the intensity of itch. Summatory score classifies 3-4 as light eczema, 4.5-7.5 as moderate eczema and 8-9 as severe eczema. Patients included in this study had ≥ 7 Rajka score. We also used the "Rule of 9" to assess the extension of eczema. Maximum extension of atopic eczema using this rule is 73.

Pathologic evaluation

Two 4 mm punch biopsies were done, in forty eight patients, on eczematous skin, before treatment and after 6 weeks treatment. Second biopsy was performed beneath the scar of the first biopsy. Tissue was fixed in formalin, blocked in paraffin and stained with haematoxilin-eosine to confirm the diagnosis of eczema.

Two morphometric parameters, epidermal area (mm^2) and epidermal thickness (mm) were useful to evaluate pathological improvement. These measurements were done by an expert pathologist, using special method semiautomatic with the image analysis Videoplan 2 (Zeiss) and the microscope Polyvar (Reichert Jury). Each area or thickness result was the media of ten epidermal area and thickness measurements. The pathologist also described the characteristics of the dermo-epidermal inflammatory infiltrate indicating the type, the distribution and the intensity.

Pattern of cytokines and cell adhesion molecule expressed locally in atopic dermatitis plays a critical role immunomodulating tissue inflammation. T cell clones type Th2 have been involved in atopic dermatitis. We have demonstrated, by *in situ* hybridization, that in acute atopic dermatitis induced by dust mite patch test, mRNA IL-4 and IL-5 but not IFN-gamma is

expressed in the lympho-histiocitary infiltrate. However very chronic and severe AD express mRNA IL-10 (14) and ICAM-1(15). We evaluate in this study mRNA IL-10 and ICAM-1 expression, by *in situ* hybridization, before and after the LA supplementation. Samples were dewaxed and treated with 5 μ g/ml proteinase K during 1 hour at 37°C. After post-fixation step using 0.4% paraformaldehyde, samples were prehybridated during 1 hour at 37°C. Sections were covered with 100 μ l/slide of prehybridation solution with biotin IL-10 (800 ng/slice) and ICAM-1 (200 ng/slice) oligonucleotide from R&D Systems. Each biopsy was tested with and without probe and polydT as control of mRNA preservation. Sections were incubated at 37°C overnight for 18 hours. Slices were washed under stringent conditions, including treatment with RNase to remove unhybridized probe. For the detection we applied the avidin/AP conjugate during 30 minutes at room temperature in chamber. After washing sections with TBS/triton five minutes two times, we applied the revealing agent during 2 hours at 37°C in the dark.

Biochemistry assessment

Four 6 mm punch biopsies were done in thirty patients, on eczematous skin and healthy skin, before treatment and after 6 weeks treatment. Posttreatment biopsies were performed beneath the scar of the first biopsies. Tissue was collected in HPLC tubes with téflon-silicone caps and 2 ml methanol. Tissue was always manipulate at 4°C and the oxygen was substitute by nitrogen in order to avoid lipid oxidation. Samples were stored at -80°C. Epidermis/dermis 5-12- and 15- hydroxyeicosatetraenoic acid (HETEs) and 13-hydroxy octadecadienoic acid (13-HODE) levels in skin biopsies were measured as indices of [EPA+DHA] and LA skin uptake and metabolism, using reverse phase HPLC.(16) Results were expressed as nanogrammes of substance per cm² of sample and gr of sample (ng/cm²/gr). 13-HODE/15-HETE index was also calculated.

RESULTS

Forty eight adult atopic patients with chronic and severe eczema (Rajka score ≥ 7) were included in this study. Sex distribution female/male was 3:1. Mean age was 24.2 \pm 1.2 (S.E.) and 6.8 (S.D.). Most patients showed family background of atopic diseases. Thirty three patients showed only atopic dermatitis and fifteen patients showed a combined atopy with eczema and other associate atopic disease. Three patients showed "Besnier prurigo" as clinic form of atopic dermatitis. IgE levels were higher than 100 UI in thirty patients ranging between 106 UI/ml and more than 5000 UI/ml. Patients often used olive oil in their diet. Only 1/3 of patients ingested n-3 fatty acid rich fish, one or two times per week.

There were no differences in AD severity among the three groups before treatment. Rajka and Rule of 9 correlation was $r = 0.9659$.

Clinic results

Clinic evaluations results showed a significant percentage reduction of both clinical scores at six weeks (Rajka score 73 \pm 10.6 SD, Rule of nine 81 \pm 9.7 SD) increasing until the evaluation made at 12 weeks (Rajka score 80.6 \pm 12 SD, Rule of nine 90 \pm 6.7 SD) in the group of patients treated with dietary supplements rich in LA.

Table I shows the percentage of reduction of both clinical scores in the three different treated groups of patients at six weeks. Fig 1 and 2 shows modifications on Rajka score and Rule of nine, after six weeks treatment in the three groups of treatment. Significant differences in the % of Rajka score and Rule of 9 reduction between patients treated with LA and [EPA+DHA] ($p < 0.0001$) and also in patients treated with LA and placebo ($p < 0.0001$) were obtained.

All patients treated with EPA+DHA and seven patients treated with placebo refused to continue the study at six weeks of treatment because itch and eczema worsening. Nauseas, vomiting and bad flavour was the most frequent complain in this group of patients. Any adverse effect was referred in patients treated with LA.

Pathologic results

We observed a marked percentage epidermal area reduction ($35.8 \pm 8.8 \text{ mm}^2 \text{ SE}$) and an important thickness reduction ($26.1 \pm 13.0 \text{ mm SE}$) in LA treated group. Table I shows the epidermal area and epidermal thickness modifications in different therapeutic groups. Histomorphometry showed significant differences in the % epidermal area reduction, between LA (Fig.3) and EPA+DHA (Fig.4) treated patients ($p < 0.005$) and between LA and placebo ($p < 0.011$). We could also observe significant differences in epidermal thickness reduction between LA and EPA+DHA ($p < 0.05$).

Chronic AD strongly express mRNA ICAM-1 in the cytoplasm of epidermal keratinocytes located around the dermal and intraepidermal inflammatory infiltrate. Very chronic atopic dermatitis express mRNA IL-10 in the cytoplasm of epidermal cells in all epidermal layers and in the inflammatory infiltrate suggesting a suppressor activity over Th1 response. After LA intake mRNA ICAM-1 and IL-10 (Fig.5) expression disappeared indicating the return of skin to physiological conditions.

Biochemistry results

We observed significant differences between the basal level of 13-HODE and 15-HETE in healthy skin ($p < 0.016$) and sick skin ($p < 0.015$). 13-HODE levels were higher indicating a possible skin physiological role.

The expected result was an increase of 13-HODE after LA intake well correlated with the reduction of epidermal proliferation. Only in healthy skin of atopic dermatitis patients, we observed an increase of 13-HODE (Table I), higher in patients treated with LA. Clinical and pathological improvement could not be statistically correlated with 13-HODE increase observed in healthy skin of atopic dermatitis patients treated with LA.

DISCUSSION

It is interesting to remember that the first studies done by Hansen showed that children with atopic eczema have reduced blood levels of unsaturated fatty acids and observed that the addition of maize oil to the diet improved the eczema.⁽¹⁷⁾ Uncontrolled trials of dietary fat supplements indicate linoleic acid to be more effective than lard.⁽¹⁸⁾ Controlled trials using small doses of linolenic acid (130 mg daily) and linoleic acid (270 mg/day) was not benefit.⁽⁴⁾ Only

high doses of LA (2880-4320 mg/day) and GLA (360-540 mg/day) are effective to treat atopic dermatitis.^(7,8)

Clinic evaluations using Rajka score and Rule of nine, pathologic assessments using epidermal area or epidermal thickness and mRNA ICAM-1 or IL-10 expression demonstrate that a vegetable sunflower oil, rich in LA and poor in GLA is also effective to treat severe and chronic atopic dermatitis in adult patients. The dosification must be high, 3 gr per day at least during 6 weeks. Animal fish oils, rich in EPA+DHA and vegetable olive oil (placebo) were ineffective to treat severe and chronic atopic dermatitis.

After LA rich oil supplies we demonstrate an epidermal area and thickness reduction then a direct or indirect action over keratinocyte proliferation. Also we observed less inflammatory infiltrate and negative mRNA IL-10 and ICAM-1 expression then a direct or indirect Th2 response immunomodulation.

Atopic dermatitis is characterized by dry skin and an impaired barrier function. It has been described the restoration of the transepidermal water loss after LA ingestion or LA topical application restauring the epidermal barrier function.⁽¹⁹⁾

It is well known that in normal skin LA sterifies skin stratum corneum ceramides, important in barrier function. It has been described an epidermal fatty acid-binding protein (E-FABP) with high affinity for fatty acids of the C18 series (in decreasing order of affinity : stearic acid > LA > oleic acid). LA and stearic acids are important components of phospholipids involved in the formation of all membranes (plasma and endoplasmic reticulum), but LA is also necessary to maintain the lipid barrier function of the skin that regulates the transepidermal water loss. Recent studies demonstrate that the decreased stratum corneum ceramides 1 (rich in LA) and 3 provoke itching and dry skin in atopic patients.⁽²⁰⁻²²⁾ LA oral supplies could contribute directly to restore epidermal barrier function.

Normal epidermis has the capacity to elongate essential fatty acids but is incapable to desaturate C18:2(n-6) and C20:3(n-6) because of the absence of activity of $\Delta 6$ an $\Delta 5$ desaturase, then skin arachidonic acid must have an extraepidermal origin.⁽²³⁾ Different studies shows increase levels of LA and decreased levels of GLA in serum, erythrocytes, fat or T lymphocytes from umbilical cord in atopic patients suggesting a defect in the $\Delta 6$ desaturation activity in this patients.⁽²⁴⁾ However, Zevenverger et al⁽²⁵⁾ consider that the hypothesis about the role of $\Delta 6$ desaturase as enzyme determinant of polyunsaturated fatty acids tissular levels is false. It is difficult to explain the efficacy of sunflower oil poor in GLA in atopic dermatitis. Perhaps an increase substrate of LA in the biochemical pathway of n-6 fatty acids to arachidonic acid could force the $\Delta 6$ desaturase enzyme activity.

Presence of 13-HODE in healthy and sick skin of atopic patients and the increase 13-HODE observed in healthy atopic skin after the ingestion of LA suggest a phisiological skin role and a possible pathogenic implication in atopic dermatitis. It could be interesting to measure also sterified 13-HODE and to study it's biological properties *in vitro*.

We conclude that vegetable n-6 fatty acids, specially LA is useful to treat atopic eczema. Good correlation between clinical and pathological improvement was observed using sunflower oil. Biological properties of each fatty acid or its metabolites need future studies. This results suggest that atopic dermatitis is initially an essential fatty acid related disease. The future research will clarify the LA mechanism or mechanisms of action on epidermal barrier function, epidermal proliferation or immunological response.

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TABLE I -
CLINICAL, PATHOLOGICAL AND BIOCHEMISTRY ASSESSMENTS

PARAMETER	LA	EPA+DHA	OO
a) <u>% Reduction of clinical scores</u>			
a.1.) Rajka Score	74.6±3.2	5.3±5.1	8.8±8.5
a.2.) Rule of Nine	81.3±3.1	-8.6±5.3	-2.7±15.0
b) <u>% Morphometric reduction</u>			
b.1.) Epidermal area	35.8±8.8	-5.4±8.9	2.6±6.4
b.2.) Epidermal thickness	26.1±13.0	-2.1±6.9	12.9±14.8
c) <u>% Increase 13-HODE</u>			
c.1.) Healthy atopic skin	186.0±92.3	13.9±60	8.4±35
c.2.) Atopic dermatitis skin	-91.6±18.2	-16.5±18.2	22.5±51

Results expressed % media± S.E.

Fig 1 . RAJKA SCORE. Modifications of " Rajka score " evaluation after treatment in the three therapeutic groups.

Fig 2 . RULE OF NINE. Modifications of " Rule of Nine " evaluation after treatment in the three therapeutic groups.

Fig.3 . Pathology. LA treated patient a) Chronic atopic dermatitis shows acanthosis with varying degrees of spongiosis. Small intraepidermal spongiotic vesicles are seen. In cases of long standing, like this case the rete ridges is greatly elongated. Dermal infiltrate consist in lymphocytes and monocytes.b) Six weeks after the treatment with L.A. supplies, skin shows a marked reduction of the epidermal area and thickness. We observe marked reduction in acanthosis and less epidermal and dermal inflammatory infiltrate.

Fig.4. Pathology. EPA+DHA treated patient. a) Chronic atopic dermatitis shows acanthosis and lympho-monocytary dermal infiltrate. b) After EPA±DHA treatment pathological changes were unmodified.

Fig.5. mRNA IL-10 expression. a) Chronic AD strongly express mRNA IL-10 in the cytoplasm of epidermal cells in all epidermal layers and in the inflammatory infiltrate suggesting a supresor activity over Th1 response.b) After LA intake mRNA of IL-10 expression disappear indicating the return of skin to phisiological conditions.

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